

An exceptional case of paternal transmission of the dark form female trait in the tiger swallowtail butterfly, *Papilio glaucus* (Lepidoptera: Papilionidae)

by

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Abstract. The melanic dark and yellow forms of the tiger swallowtail butterfly, *Papilio glaucus glaucus* L., are believed to be controlled by a locus on the Y (W) chromosome. Since the female is the heterogametic sex (XY) in Lepidoptera, dark females should (and generally do) produce only dark daughters while yellow females produce only yellow daughters. Exceptional broods have been reported in which some yellow females arise from dark, and more rarely some dark females arise from yellow mothers. Scriber et al (1986) have shown that these results (as well as both colors of females arising from either colored mother) can be obtained experimentally by hybridizing and backcrossing with the northern subspecies *Papilio glaucus canadensis* R & J.

The purpose of this communication is to describe the results of a highly unusual case in which the locus for the dark gene controlling melanism from a dark female *P. glaucus* was transmitted by a male in two separate pairings. This observation has never before been reported and is significant that it suggests that the locus for black color is not necessarily totally lost when it (rarely) dissociates from its normal (Y) chromosome. Since chiasmata at oogenesis in female Lepidoptera are generally believed to be non-existent, crossing-over is believed not to occur in female Lepidoptera. While our results do not permit us to distinguish between a cross-over event and a non-disjunction of the sex chromosome, we nonetheless have observed results of a rare event, especially for Lepidoptera.

Introduction:

The melanic dark and yellow forms of the tiger swallowtail butterfly, *Papilio glaucus glaucus* L., are thought to be controlled by a locus on the Y (W) chromosome (Clarke and Sheppard, 1959, 1962). In fact, this locus controlling dark morph expression in female *P. glaucus* is one of

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but a few sex-linked marker genes in butterflies (Robinson, 1971; Soumalainen, 1973; R. Hagen, 1986, and pers. comm.). The female is the heterogametic sex in Lepidoptera, and dark females should (and generally do) produce only dark daughters, and yellow females produce only yellow daughters. Exceptional broods have been reported (Edwards, 1884; Clarke and Sheppard, 1959, 1962; Scriber et al., 1986) in which some yellow females arise from dark, and more rarely some dark females arise from yellow mothers. Scriber et al. (1986) have shown that these results (as well as both colors of females arising from either colored mother) can be obtained experimentally by using hybrids with the northern subspecies *Papilio glaucus canadensis* R & J. Intermediate colored females with a "peppered" or "sooty" color over the yellow tiger-striped background are also observed in nature (see Edwards, 1884; Clark and Clark, 1951), and have been experimentally produced by hybridization or backcrossing with *P. rutulus* or *P. g. canadensis* (Clarke and Willig, 1977; Clarke and Clarke, 1983; Scriber et al., 1986).

In addition to the partial or complete suppression of the Y-linked melanism in female *P. glaucus* when paired to *P. rutulus* and *P. g. canadensis* males (Scriber et al., 1986), it has also been suggested that, if the Y chromosome bearing the locus for the dark gene is occasionally lost during meiosis of dark females, yellow daughters would be produced (Clarke and Sheppard, 1962; Clarke et al., 1976). Scriber et al. (1986) describe such a case in which loss of the locus for dark color in \bar{F}_2 hybrid females is likely to have occurred. However these authors have also observed cases of yellow F_1 hybrid daughters of dark mothers which retain the locus for black color. Depending on the male used in subsequent matings, all yellow, all black, or both colors can be obtained from these yellow hybrid or yellow backcross females (Scriber, 1985; Scriber et al., 1986).

Here we describe the results of a highly unusual case in which the locus for the dark gene controlling melanism in female *P. glaucus* seems to have been transmitted by a male to two different hand-pairings. This situation has never before been reported and is significant in that it suggests that the locus for black color is not necessarily totally lost when it (rarely) dissociates from its normal (Y) chromosome. Since chiasmata at oogenesis in female Lepidoptera are generally believed to be absent, crossing-over is believed not to occur in female Lepidoptera (Haldane, 1922; Robinson, 1971; Clarke and Sheppard, 1973; Soumalainen et al., 1973; Turner and Sheppard, 1975). However a suspected crossover in the supergene controlling female polymorphism in *Papilio memnon* L. has been reported (Clarke and Sheppard, 1977).

Our recent studies of the genetic basis of dark morph expression in *Papilio glaucus* have involved hand-pairings and mass-rearing of thousands of individuals derived from various geographic locations across North America (Scriber and Evans, 1986a). All of these and the

following specimens are maintained in the *Papilio* research collection of J.M.S. at the Department of Entomology at Michigan State University.

Results.

A near-normal but slightly melanic or "sooty" (Fig. 1) yellow male adult butterfly was obtained from a normal-appearing dark morph mother (#674) which was field-captured in Adams County, Ohio by M. H. Evans and W. W. Warfield on July 1983. This male eclosed in 1984 and was hand-paired on May 14, 1984 (#1129) to a virgin yellow morph female (*P. g. glaucus*) which was lab-reared from a yellow morph Ohio female (#631) field-captured on 14 May, 1983. On the following day (May 15, 1984) a second pairing (#1132) using the same male was made to a virgin *P. g. canadensis* female (which was lab-reared in 1983 from a 25 June, 1983 field-captured yellow female from Barron County, Wisconsin). All subsequent larvae were reared through to pupation under identical controlled environment conditions (16/18 photo/scotophase, corresponding thermoperiod of 23 ½°C/19 ½°C). Pupae were weighed and adults were permitted to eclose in cylindrical screen cages.

To our surprise, we observed dark as well as the expected (yellow) females in the progeny of both crosses (Table 1). According to all understanding to date, dark females were not expected to occur in offspring of either of these pairings. We have never before observed dark daughters in the lab-reared offspring of more than 600 different *P. g. canadensis* mothers. While it is possible that *Papilio glaucus* larvae could be accidentally introduced with foodplant leaves in our laboratory mass rearing procedures at Madison, this is unlikely and careful precautions are continually made to prevent this possibility. We

Table 1. Special case in which an aberrant-colored male mated to two different yellow females resulted in dark female daughters (Madison, WI, 1984).

Parentage*	Mating number	Total pupae reared (n)	Males eclosed (1984)	Males eclosed (1985)	Females eclosed			Pupae remaining	
					Yellow (1984/1985)	Dark (1984/1985)		Dead (n)	Alive (1986)
OH(Y) × OH(D)*	1129	69	32	2	9	3	16	1	2
Pgc × OH(D)*	1132	82	40	4	12	0	21	0	3

* Female parent listed first. The OH(D) parent represents an aberrant-colored male (see Fig. 1a and 1b) reared in 1983 from a normal appearing dark morph mother (#674) captured in Adams County, Ohio on 8 July, 1983. This male was mated to a yellow daughter of an Ohio *P. g. glaucus* yellow female #632 on 14 May, 1984 (mating #1129); and to a daughter of *P. g. canadensis* female #614 on 15 May, 1984 (mating #1132).

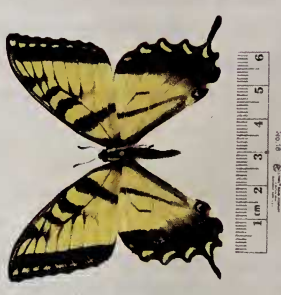
have not observed any such occurrences in the last 5 years with nearly 120,000 ova in our lab. Such errors cannot possibly account for the 38 dark females produced from these two pairings.

Discussion.

We interpret the results as evidence of male transmission of the gene controlling black color (which is found on the Y chromosome of the heterogametic female). The sons of pairings 1129 and 1132 were all normal in appearance (i.e. they were not black or dark colored as the female morph can be). Approximately 2/3 of the daughters were dark morph and 1/3 yellow morph, and none of the daughters exhibited partial color or mosaic patterns (e.g. dark with irregular blotches/patches of yellow background showing; see Scriber et al, 1986). This suggests that all cells of dark daughters contain the gene for black color, and favors the idea of non-disjunction or a cross-over of this locus, rather than a particulate cytoplasmic explanation (see Clarke and Sheppard, 1959, 1962).

FOLLOWING PAGE CAPTION:

- Fig. 1. Offspring of dark female #674 from Adams Co., Ohio, 1983: a) dorsal and b) ventral of "slightly aberrant" male (wt. 1.1642); c) dorsal and d) ventral of a "normal" sibling (wt. 1.0459). This first (aberrant) is the male parent in crosses 1129 and 1132 (see Table 1), and is our suspected "carrier" of the female melanism locus.
- Fig. 2. F₁ hybrid offspring of pairing #1132 (a virgin daughter of a 1983 Barron Co., Wisconsin *P. g. canadensis* female × the aberrant male, wt. 1.1642, of Fig. 1a & b). a) dorsal and b) ventral of a "slightly aberrant" male (wt. .9386) and c) dorsal and d) ventral of a normal sibling male (wt. .8522).
- Fig. 3. Offspring of an F₂ pairing (#1695; see Table 2) of a dark daughter and a slightly aberrant male (shown in Fig. 2a, 2b) both derived from pairing #1132 (Table 1). a) dorsal and b) ventral of an aberrant male (wt. .9410), and c) dorsal and d) ventral of a normal male sib (wt. 1.0416).
- Fig. 4. Female offspring of a cross between a yellow morph *P. g. glaucus* × the "aberrant" male (cross #1129): a) dorsal and b) ventral of a typical dark morph, (wt. 1.4010) and of a typical yellow morph c) dorsal d) ventral (wt. 1.3700) sibling (see Table 1).
- Fig. 5. Female offspring of F₁ hybrid cross of a *P. g. canadensis* × "aberrant" male *P. g.* (cross #1132). a) dorsal and b) ventral of a typical dark morph (wt. 0.6964), and c) dorsal and d) ventral of a typical yellow morph (wt. 0.6755) sibling (see Table 1).
- Fig. 6. A wild collected "aberrant" male from Dane County, Wisconsin (collected 10 August 1983).





Under the hypothesis of a non-disjunction as a causal mechanism, we could expect our male to be of the genotype X (XY^D), where the "Y" represents the Y chromosome carrying the gene for dark color. The *P. g. canadensis* and yellow morph *P. g. glaucus* females would both be of the genotype XY, and offspring (1129 and 1132; Table 1) would be expected to be the following: XX and X (XY^D) males, XY yellow females, and Y (XY^D) dark females. This explanation would account for the occurrence of both dark and yellow female daughters; however so would the hypothesis of a cross-over event.

In a cross-over of the locus for dark color in this species we would expect the male parent of 1129 and 1132 to be of the genotype $X^D X$. When paired to the *P. g. canadensis* female and the yellow morph *P. g. glaucus* female (both presumably XY genotype) we would expect the following: XX^D and XX males, XY yellow females, and $X^D Y$ dark females.

Both the cross-over hypothesis and the non-disjunction hypothesis provide explanations for observed yellow and dark daughters. However, neither explains the observed deviation from an expected 50:50 ratio of female morphs. Similarly, the reason for the melanism being restricted to only (some) daughters and none of the sons of this male carrier (not expressed in himself or his sons) is unresolved for both hypotheses. We did, however, notice a slight "sootiness" or semi-melanism in the generally normal tiger-striped yellow background proximally on the dorsal surface of the wings in this original male parent (Fig. 1) and in one of his 44 sons (Fig. 2). We had hoped that this could prove to be a phenotypic marker for the male black locus carriers reflecting the X (XY^D) or the XX^D genotype (from either a non-disjunction or a crossover, respectively).

Subsequent pairings with offspring of pairings 1129 and 1132 (Table 1) have yielded poor results. Nonetheless, when the aberrant male son (shown in Fig. 2) was mated to one of his sisters (pairing 1695; Table 2) one of the resulting 5 male F_2 hybrid sons was markedly melanic in the proximal 1/3 of the wings (Fig. 3a, b).

Female offspring resulting from pairings #1129 (*P. g. glaucus* \times *P. g. glaucus*) and #1132 (*P. g. canadensis* \times *P. g. glaucus*) are typical dark or typical yellow in color pattern (Figs. 4 and 5) with one exception, where one daughter is a "dark intermediate". It should be noted that the female progeny of cross 1129 are larger than those of 1132, reflecting the genetic differences in size between *P. g. canadensis* and *P. g. glaucus*. It is also noteworthy that the dark females of cross #1132 represent the only known case of melanism being expressed in F_1 hybrids from a *P. g. canadensis* mother (Fig. 5).

In an attempt to follow up the genetic explanation of our unique results in pairings 1129 and 1132, (Table 1), we hand-paired male, yellow female, and dark female offspring of both crosses. Twenty-one different yellow and dark females from cross 1132 were hand-paired

Table 2. A 1984 F₂ hybrid pairing of a dark female and slight aberrant male (both from pairing 1132; Table 1).

Parentage	Mating number	Total pupae reared	Males eclosed		Females eclosed				Pupae alive (1985 October)
					Yellow		Dark		
			(1984)	(1985)	(1984)	(1985)	(1984)	(1985)	
(1132*) ²	1695	10	2	3	1	0	1	1	2
* Male with aberrant color; dark morph female									

Table 3. Pairings of a yellow female F₁ hybrid and two of her dark daughters (Madison, WI; 1985).

Parentage	Mating number	Total pupae reared	Males eclosed		Females eclosed				Pupae alive (1985 October)
					Yellow		Dark		
			(1984)	(1985)	(1984)	(1985)	(1984)	(1985)	
1129(Y) × Pgg* *wild WI male	2343 (see below)	204	—	37	—	6	—	90	71
2343(DK) × Pgg* *wild OH male	2957	88	—	35	—	0	—	37	16
2343(DK) × Pgg* *wild OH male	2974	81	—	35	—	0	—	36	10

(with copulations of 30 minute durations or more). These pairings resulted in only 8 females which produced eggs, only two of which produced any larvae (#1695 produced 34 larvae, #1542 produced 1 larva). The single most useful cross of these attempts was #1695—an F₂ hybrid of a dark female from 1132 × her aberrant male sibling; see Figs. 2a, 2b. This cross generated both yellow and dark daughters as expected under the crossover/non-disjunction hypotheses (Table 2). Since none of his normal-type siblings (see Figs. 2c, 2d) produced female daughters from fourteen mating attempts, we are unable to evaluate whether this aberrant color in males is indicative of possession of the female melanism gene (i.e. a “carrier” criterion).

Seven different females from cross #1129 were also hand-paired, of which only 3 produced eggs and only one (pairing #2343 in 1985) produced any larvae. This backcross of a yellow morph female (from 1129) to a wild Wisconsin *P. g. glaucus* male resulted in 310 larvae, which produced 204 pupae. Unfortunately, instead of resolving the genetic explanation of the paternal transmission of the melanism

capacity, cross #2343 has become an enigma. This cross involving a yellow mother produced 90 dark daughters, 6 yellow daughters and 37 sons (Table 3). The existence of dark daughters was totally unexpected under our hypotheses of crossover and/or non-disjunction because this female parent (XY) should have produced only yellow daughters. Two subsequent pairings of her dark daughters (#2957 and #2974; also in 1985) to wild male *P. g. glaucus* from Ohio yielded the expected all dark female offspring and an equal sex ratio (Table 3; cf Scriber et al, 1986).

We had hoped that the matings in the 1985 season would clarify our suspected crossover/non-disjunction hypotheses, but this was not the case. At present, we have no explanation for the appearance of dark daughters in pairing 2343 (Table 3). The yellow mother from cross 1129 (Table 2) would presumably have been dark if she possessed the gene for melanism, since any autosomal suppressor in *P. g. canadensis* would not be involved in any pure *P. g. glaucus* lineage (Scriber et al., 1986). However, we are not absolutely certain that the Adams County (Ohio) population is free of *P. g. canadensis* genes from the Appalachian Mountain region (e.g. Ritland and Scriber, 1985; Scriber and Hainze, 1986).

Conclusions.

We must emphasize that although we cannot prove a crossover or non-disjunction event, we nonetheless have observed the transmission by a male of the dark morph trait to his daughters (from a mating with a Wisconsin *P. g. canadensis* yellow female, and from a mating with an Ohio *P. g. glaucus* yellow female). We do not feel that this phenomenon (appearance of dark daughters from yellow mothers of two different subspecies) is likely to be explained by autosomal melanism suppressor effects from *P. g. canadensis* introgression. This would require that both the yellow Ohio female and the yellow northern Wisconsin female (the female parents in Table 1) were the result of *P. g. canadensis* introgression into an ancestrally dark stock. This possibility may not be as farfetched as initially assumed (see Scriber and Evans, 1986a and 1986b). Another remote explanation is that the wild Wisconsin male used in pairing #2343 was simply another independent example of a crossover/non-disjunction, which would also explain dark daughters from a yellow mother presumed to lack the gene for melanism. In this regard it is interesting that partially melanic males (e.g. Fig. 6; and compare Figs. 1, 2, and 3) have been captured from the same population in Wisconsin as the mated male in cross 2343. None have been tested for the dark gene transmission potential however.

Should we be correct in assuming that our results reflect some form of crossover in female Lepidoptera, then there should be special precautions taken by systematists who employ maternal DNA (maternal inheritance of DNA) techniques in evaluating phylogenies, and assume a clear record of the maternal lineage (see Avise and Lansman, 1983

for further discussion). The adaptive significance of achiasmatic meiosis (and the assumption that this is accompanied by the absence of crossing-over) are not entirely clear, but it has evolved repeatedly in at least 10 major groups of animals (White, 1973). Sexual mosaics, color mosaics, and bilateral gynandromorphs of *Papilio glaucus* may be more common than generally believed, especially near suspected hybrid zones (Clarke and Clarke, 1983; Scriber and Evans, 1986b). Perhaps such chromosomal/developmental abnormalities will provide us with other additional opportunities to evaluate our crossover/non-disjunction hypotheses in the future.

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